

Aging & Rehabilitation

An Interdisciplinary Research Seminar Series



Sponsors

Department of Veteran Affairs

- Rehabilitation Outcomes Research Center (RORC)
- Brain Rehabilitation Outcomes Research Center (BRRC)
- Geriatric Research, Education, and Clinical Center (GRECC)

UF College of Medicine

- Institute on Aging
- Department of Aging and Geriatric Research

UF College of Public Health and Health Professions

- Brooks Center for Rehabilitation Studies

UF College of Liberal Arts and Sciences

- Center for Gerontological Studies

UF McKnight Brain Institute

UF College of Nursing

Schedule

- August 29th, 2005 – May 22nd, 2006
- Mondays, 12:00 – 1:00
- HPNP Room – G103

CYBER SEMINAR VENUES

- VA RORC, Conference Room, Suite 350
- VA BRRC, VA Nursing Home, Room 271-12
- UF Brooks Center Conference Room, Jacksonville

Themes

- Basic Science (C. Leeuwenburgh)
- Clinical Science (R. Beyth)
- Outcomes / Health Policy (E. Andresen)
- Behavioral and Social Research (M. Marsiske)
- Cutting Edge / New Research (T. Foster/ J. Aris)

How Do Cells Age?

John P. Aris, Ph.D.

Associate Professor

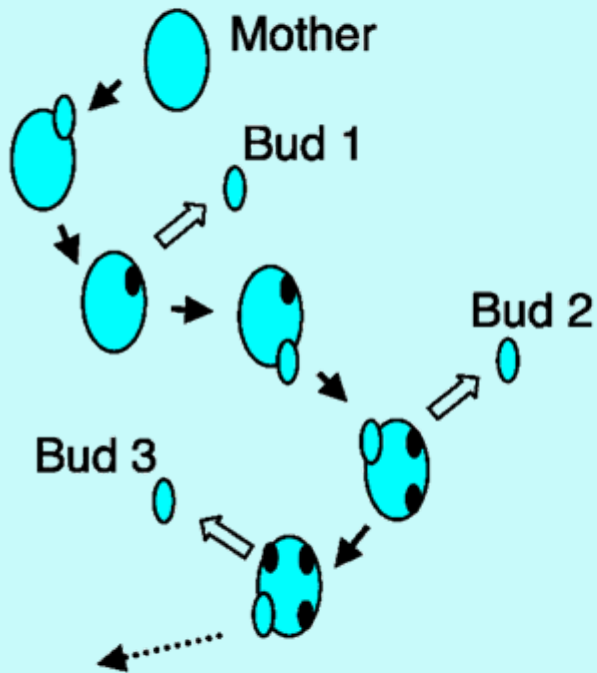
Department of Anatomy and Cell Biology

Cell Aging - One Level Among Many

Level	Theory of Aging
Molecule	Metabolic error
Cell	Free radical (molecular damage) Somatic mutation
Tissue	Gene dysregulation Genome integrity
Organ	Defect accumulation Cell senescence
Organ system	Neuroendocrine Immunological
Individual	Disposable soma Antagonistic pleiotrophy
Population	Post-reproductive selection shadow

Replicative and Chronological Aging

Aging in the yeast *S. cerevisiae*
is the number of buds a
mother cell produces



REPLICATIVE AGING
of 1 MOTHER CELL

Aging in the worm *C. elegans*
is the number of days
a worm lives



↓
Prod individuals daily
to check for the ability
to move any part of the body

↓
CHRONOLOGICAL AGING
of 959 MOTHER CELL

Hekimi &
Guarente,
2003,
Science.
299:1351

Aging and Senescence in Cells

Aging in Cells

Changes during "normal" life span that are:

Universally observed in species

Not due to a disease process

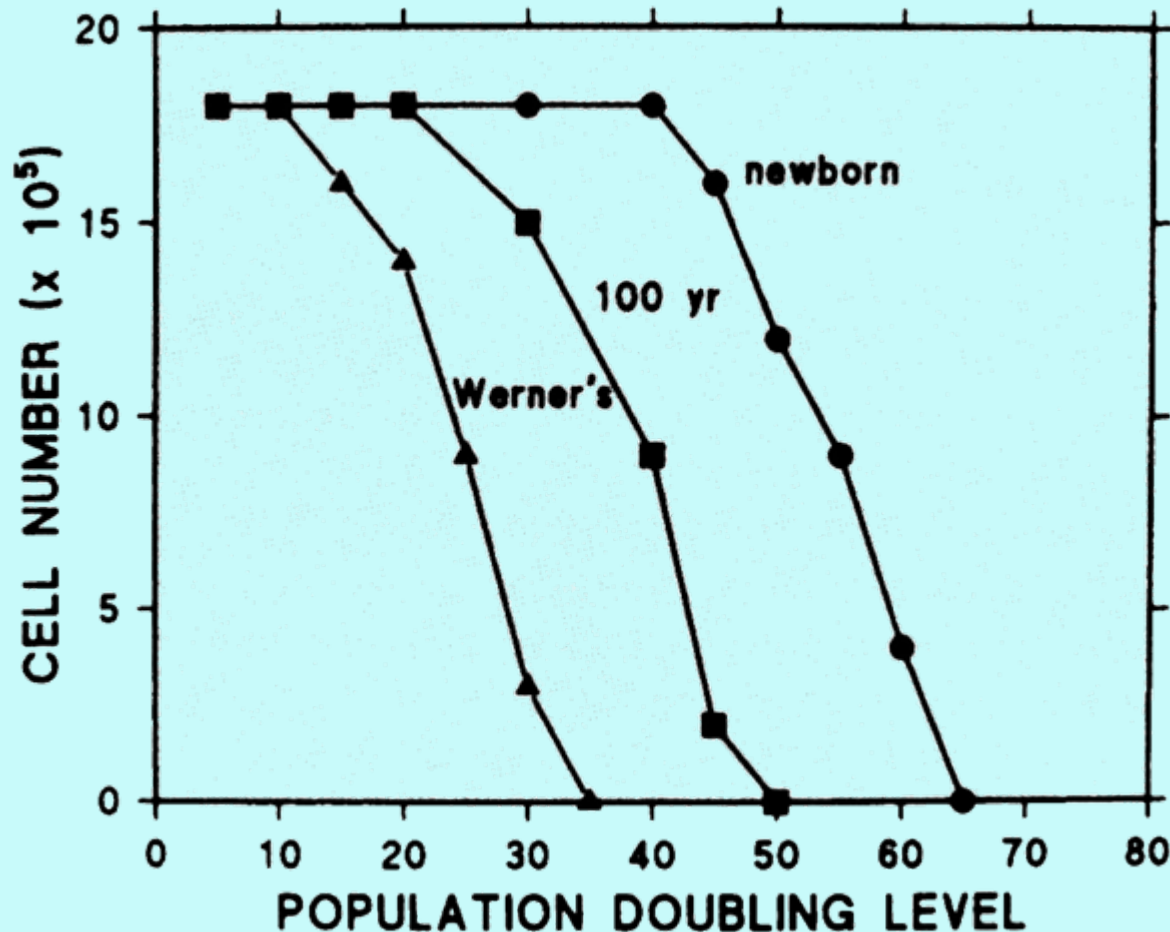
Usually irreversible

Progressive

Cell Senescence

Stage at which replicating cells are viable but no longer capable of cell division as a result of changes due to aging

Replicative Aging in Human Cells



Human fibroblasts from skin were grown through many population doublings in tissue culture until senescence.

Dice, 1993, *Physiol. Rev.* 73:150

Human fibroblasts in culture exhibit a “Hayflick” limit (Hayflick & Moorhead, 1961, *Exp. Cell Res.* **25**:585)

Hayflick Limit

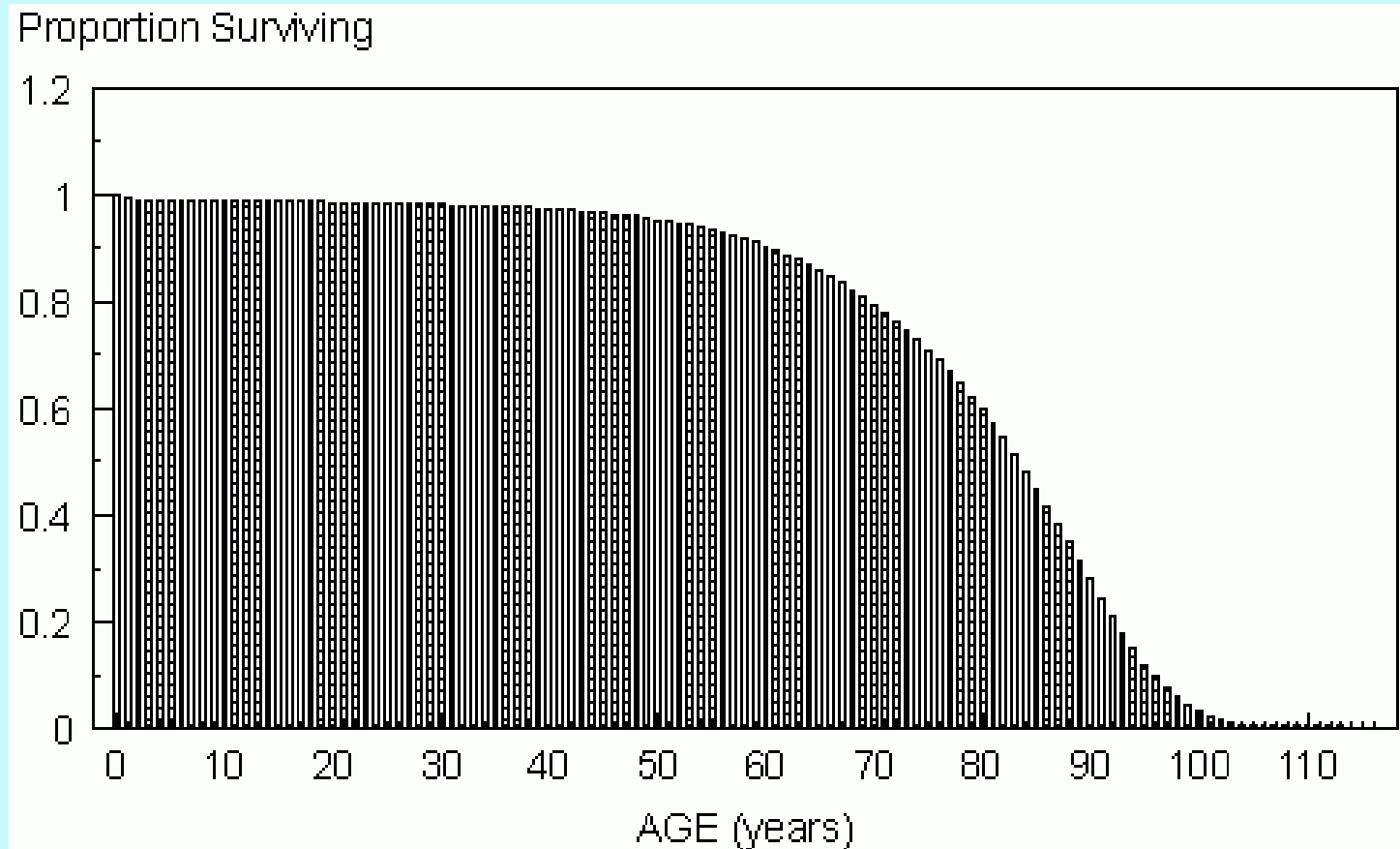
Hayflick limit - number of cell population doublings that are required to reach replicative senescence

Hayflick limit is a measure of replicative aging

Hayflick limits have been observed and measured in many cultured cell types from many different species

Hayflick limit for cells in culture correlates with the organism's chronological life span in many cases

Gompertz Law of Mortality



Gompertz formula - relationship between age and mortality

Exponential increase in mortality rate with age

Similar mortality curves are characteristic of aging in cells

Werner Syndrome



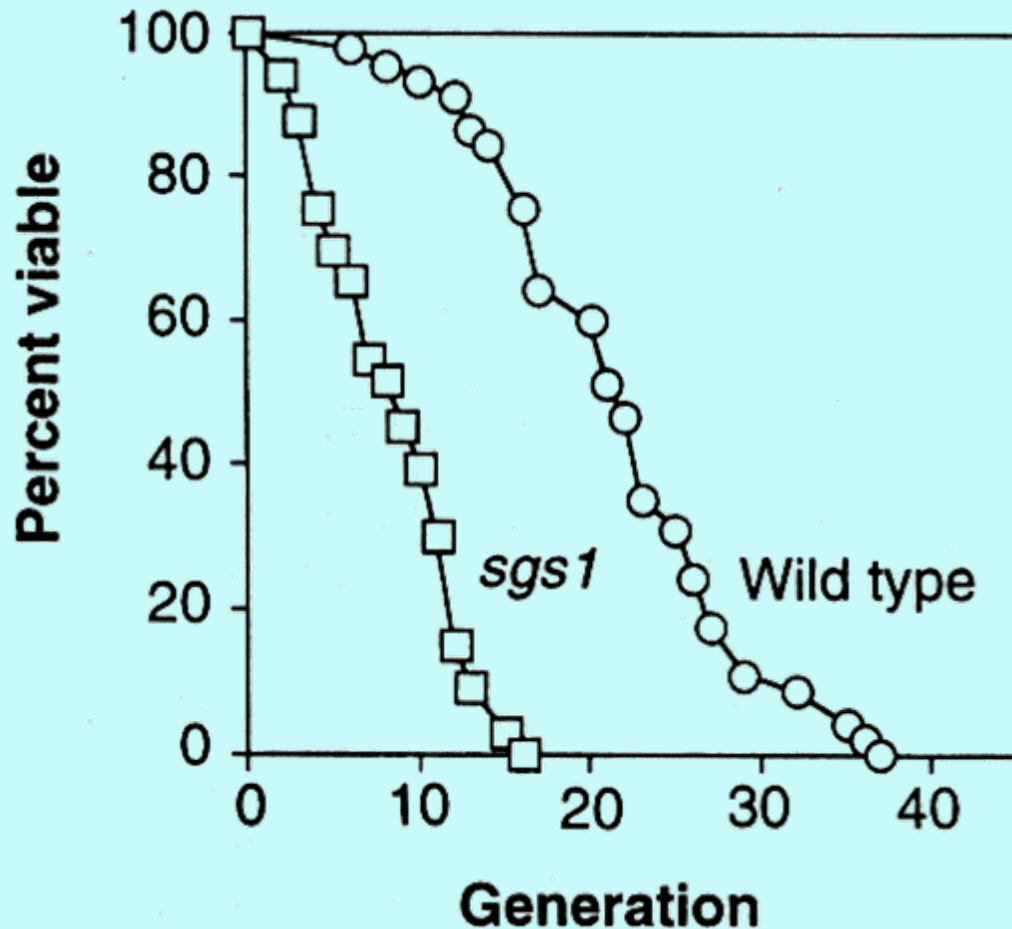
A Japanese-American Werner patient as a teenager and at age 48. She had eight children, two of whom were also affected. At 48, she had hair loss and graying, thin extremities, atrophy of the skin, among other symptoms. She lived longer than most Werner patients, dying at 57. (Epstein et al, 1966, *Medicine* **45**:177)

Werner gene was identified in 1996
(Chang-En et al, 1996, *Science* **272**:258-262)

WRN encodes a RecQ family DNA helicase

In vivo function remains unknown

Replicative Aging in Yeast

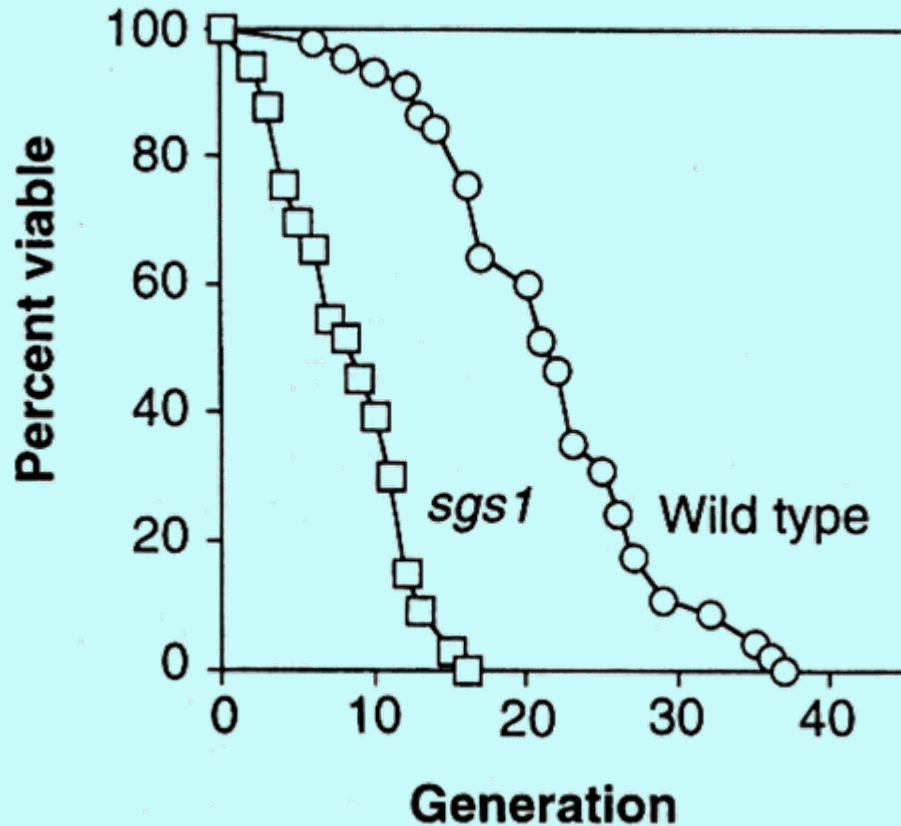


SGS1 is the yeast homolog of *WRN*

sgs1 mutants have a short life span and show signs of aging (sterility and genomic instability)

Sinclair et al, 1997, *Science* **277**:1313

Replicative Life Span in Yeast



Sinclair et al, 1997,
Science **277**:1313

Replicative life span is limited and declines exponentially
SGS1 is the yeast homologue of human Werner (*WRN*) gene
sgs1 mutants show signs of aging (sterility, genomic instability)

Centenarians in Sardinia



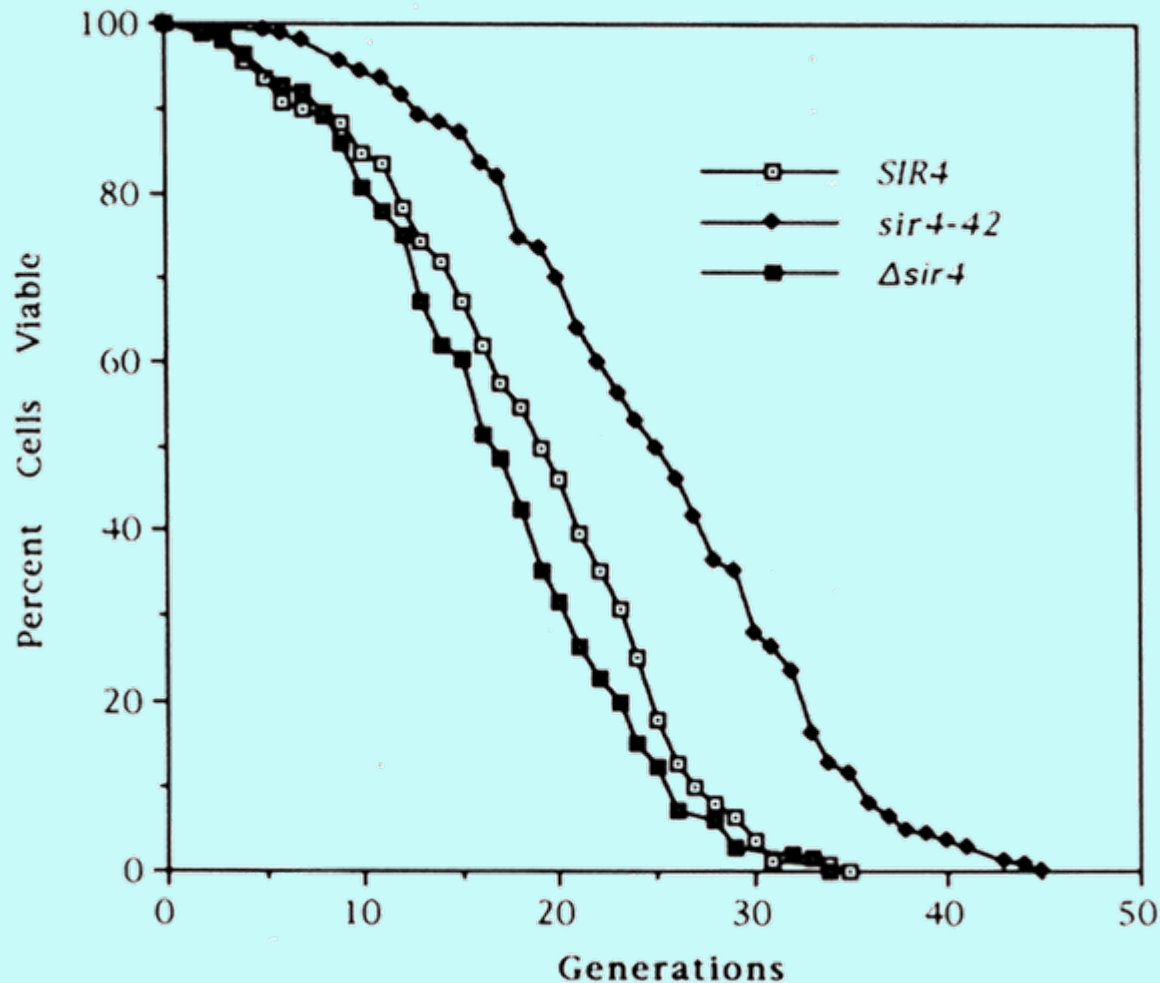
Koenig, 2001,
Science **291**:2074

Sardinia has highest proportion (1:1) of male centenarians

Worldwide ratio of women to men who live to 100 is 5:1

Genetic profiling of centenarians is underway

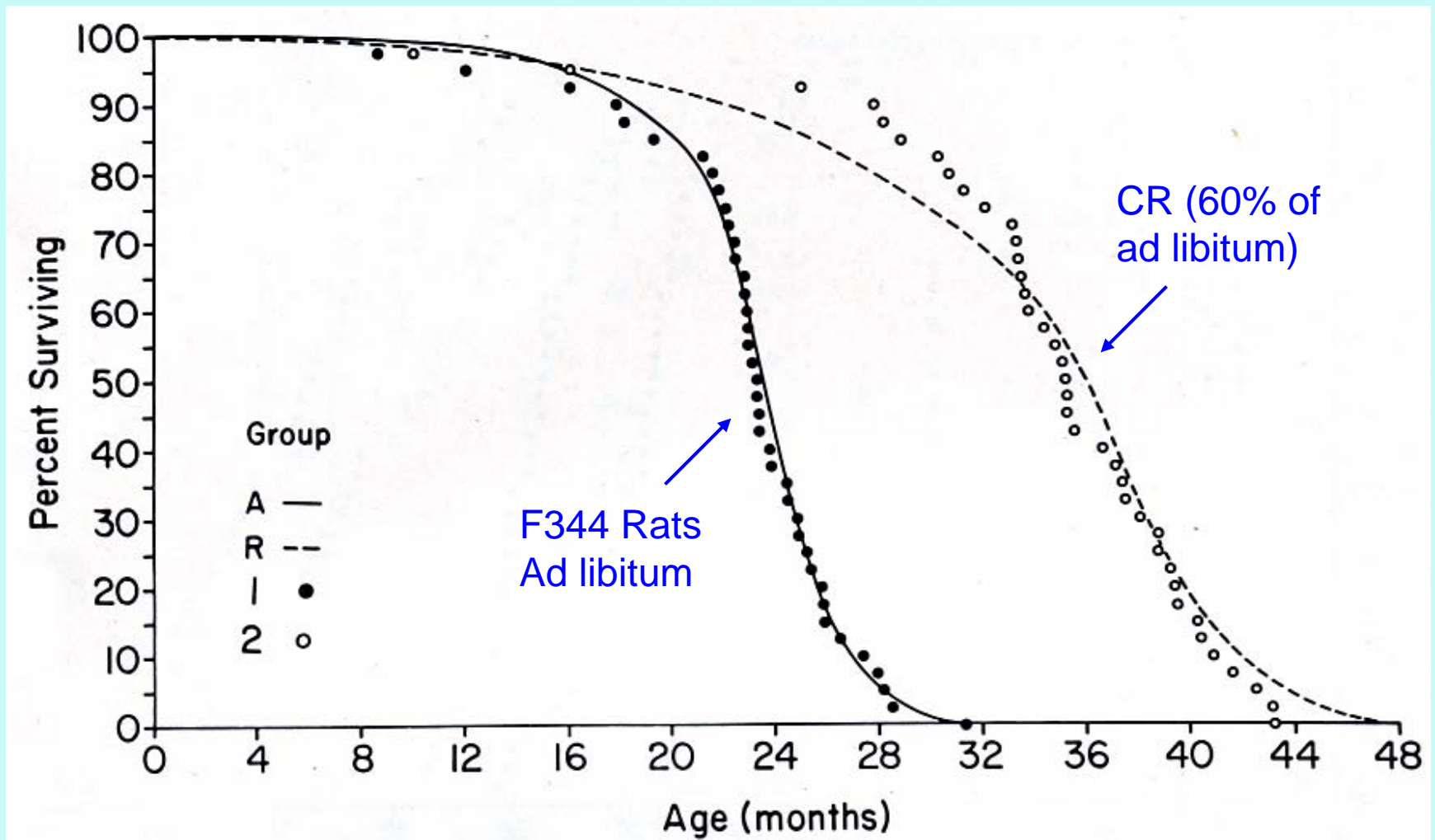
Longevity Genes



Kennedy et al,
1995, *Cell* **80**:485

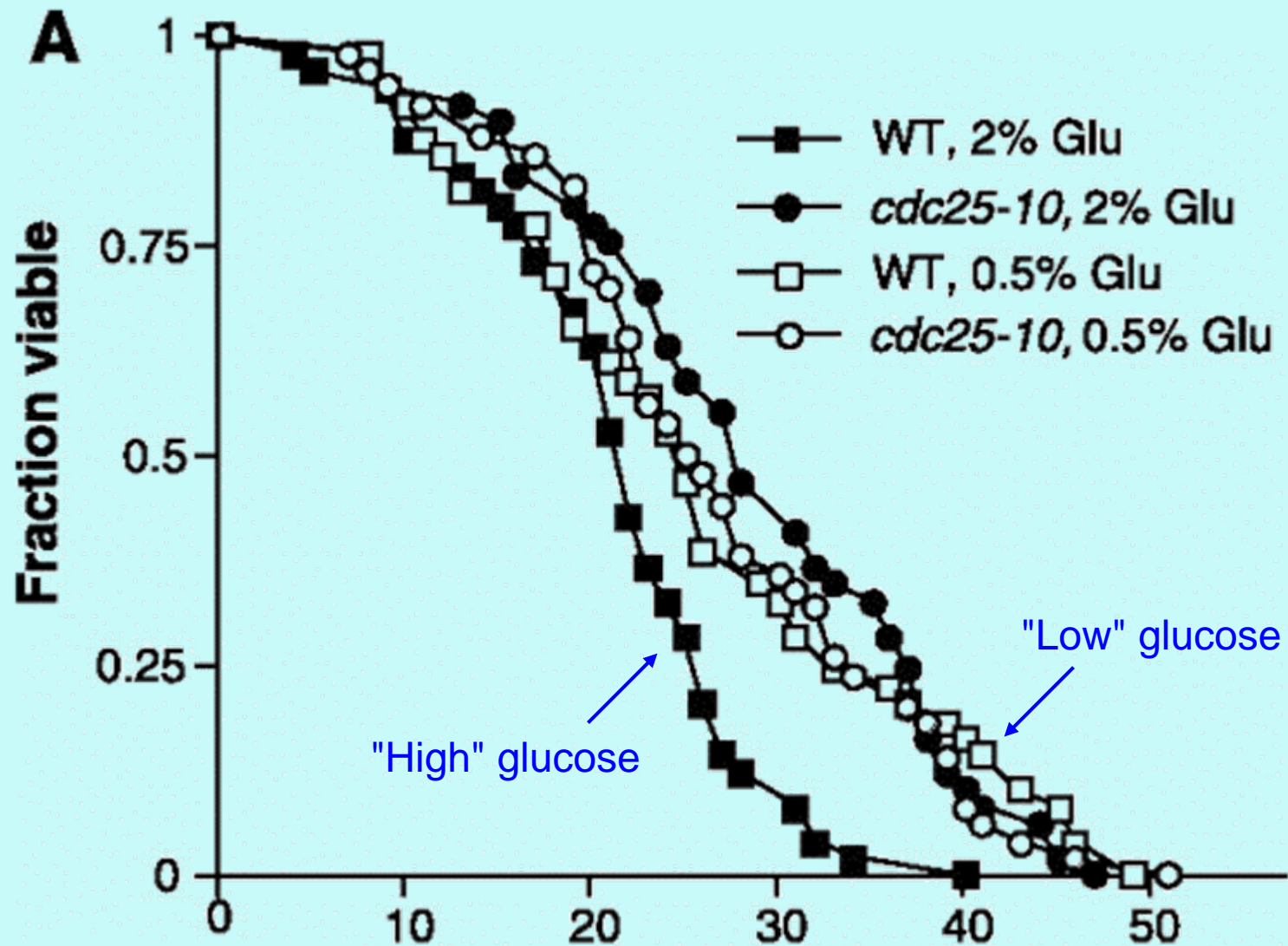
Yeast longevity gene - *UTH4* (*SIR4-42*) is a semi-dominant gain-of-function allele that extends replicative life span

Caloric Restriction (CR)



Yu et al., 1985, *J. Gerontol.* **40**:657

Caloric Restriction (CR)



Lin et al., 2001, *Science* **289**:2126.

Cell Aging

- **Cell life span (replicative & chronological) is limited**
- **Cell life span exhibits Gompertz-like statistical behavior**
- **Cell life span is subject to genetic regulation**
- **Cell life span is regulated by single genes (suggesting that life span is controlled by a hierarchical regulatory network with a limited number of control points)**
- **Cell life span can be extended by caloric restriction**

Goals for Studies of Cell Aging

- **Understand molecular processes that underlie aging**
- **Identify genes that specifically influence aging**
- **Understand pathways that regulate aging**
- **Devise strategies to delay (prevent?) aging**

Theories of Aging

Metabolic error - inherent error rate in complex biological processes (e.g., transcription, translation) increases with age and leads to decline in fidelity of these processes

Free radical - molecular damage, primarily due to reactive oxygen species (ROS), cause progressive loss of function

Somatic mutation - mutations in DNA due to damage accumulate progressively and are inherited by progeny cells

Gene dysregulation - changes in patterns of gene expression, due to damage or a genetic program, cause aging

Genome integrity - maintenance of genomic integrity is a balance between pro-aging and pro-longevity processes

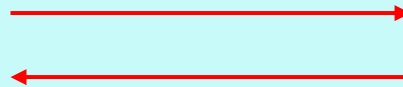
Defect accumulation - errors accumulate and lead to cell dysfunction and senescence (normal "wear and tear" theory)

Metabolic Error Theory

Young

Complex process
(transcription,
translation)

Inherent
error rate



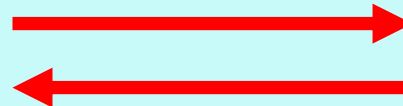
Gene products
required for
complex process

Impaired
function

Old

Complex process
(transcription,
translation)

Inherent
error rate



Gene products
required for
complex process

Impaired
function

Free Radical Theory

Damage to cells and their parts is due to free radicals

Damage to cells accumulates over the life span

Damage results in cell dysfunction and senescence

Free Radicals

Free radical - molecule or atom with unpaired (odd) electron(s)

Free radicals are highly reactive and promiscuous

Free radicals form covalent bonds with other molecules (i.e., form adducts that can block or impair function)

Free radicals initiate chain reactions that are propagated as molecules react with others in an attempt to pair electrons

Free radical damage to biological molecules, proteins and lipids and others, has been shown to take place during aging

Free radicals are formed by endogenous (e.g., metabolic) and exogenous mechanisms (e.g., X-rays, UV)

Reactive Oxygen Species (ROS)

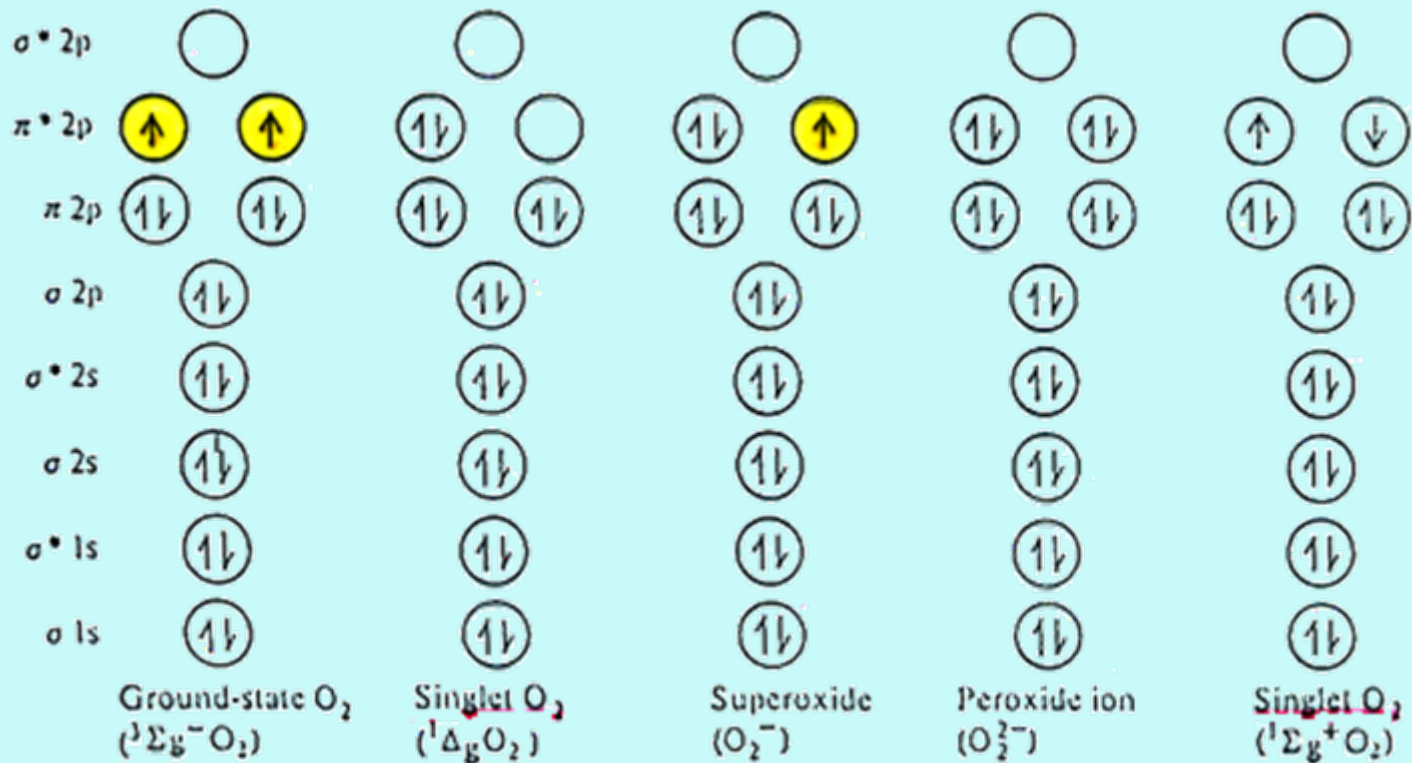
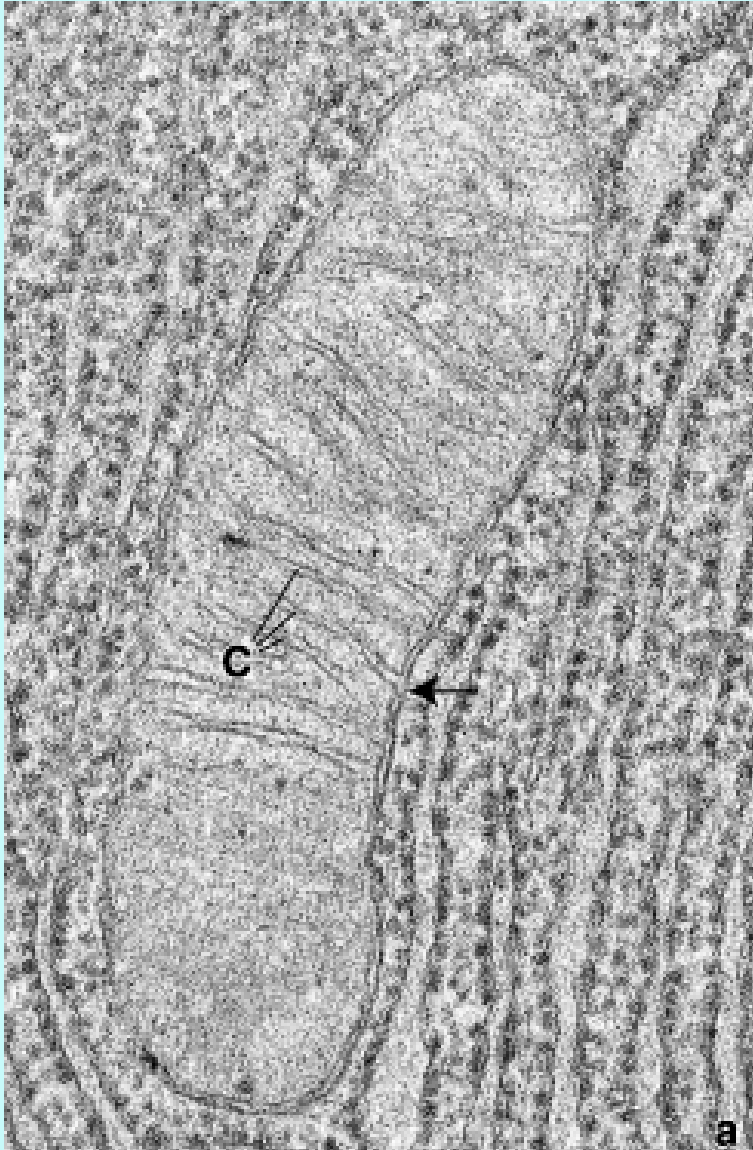


Fig. 1.6. Bonding in the diatomic oxygen molecule.

Most free radicals in cells are formed from oxygen
 Superoxide (O_2^-) and peroxide (O_2^{2-}) are highly reactive
 Hydrogen peroxide (H_2O_2) is membrane permeant

Mitochondrion



Function

ATP production

Heat production

Metabolic precursor synthesis

Oxidation of fatty acids

Structure

Outer membrane (OM)

Inner membrane (IM), cristae

Intermembrane space

Matrix

Genetics

mtDNA - encodes 13 proteins
tRNAs, and rRNAs in human

Most (>98%) proteins are
encoded by nuclear genes

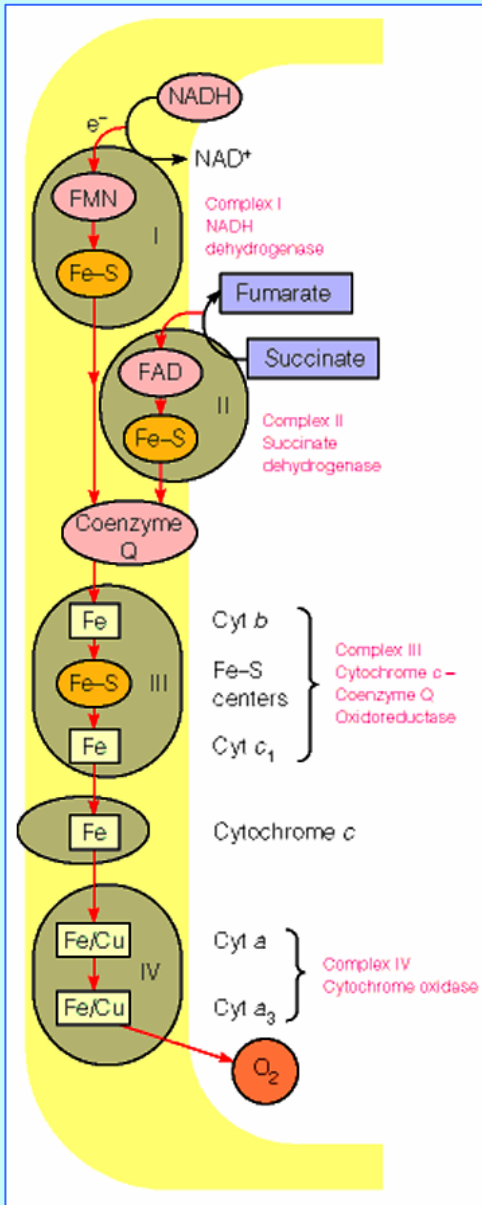
Aerobic Respiration in Mitochondria

Oxygen is final electron acceptor in aerobic metabolism in mitochondria

~95-99% of O_2 is reduced by cytochrome c oxidase complex to form metabolic H_2O

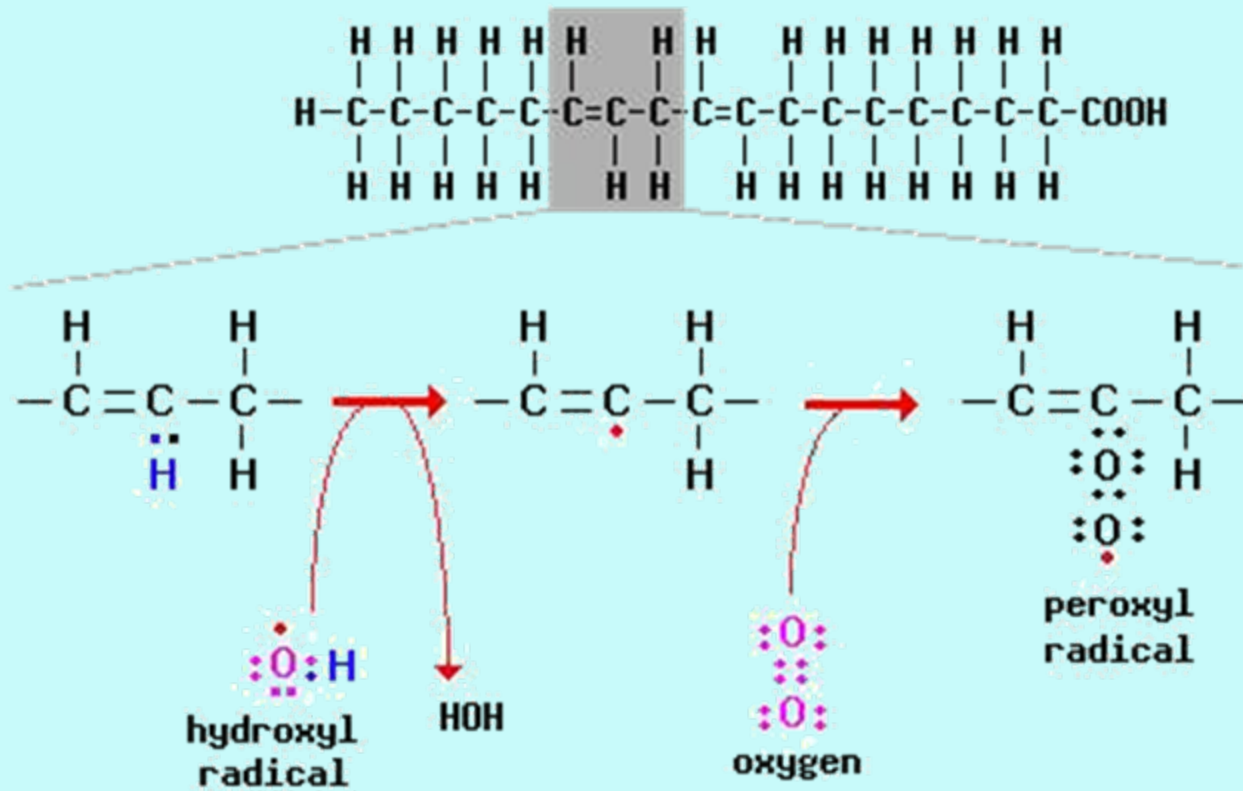
~1-5% of O_2 forms ROS

Mitochondria are prone to ROS damage - most ROS form in mitochondria, where they react with mtDNA, lipids, proteins



Electron transport
chain in mitochondrial
inner membrane

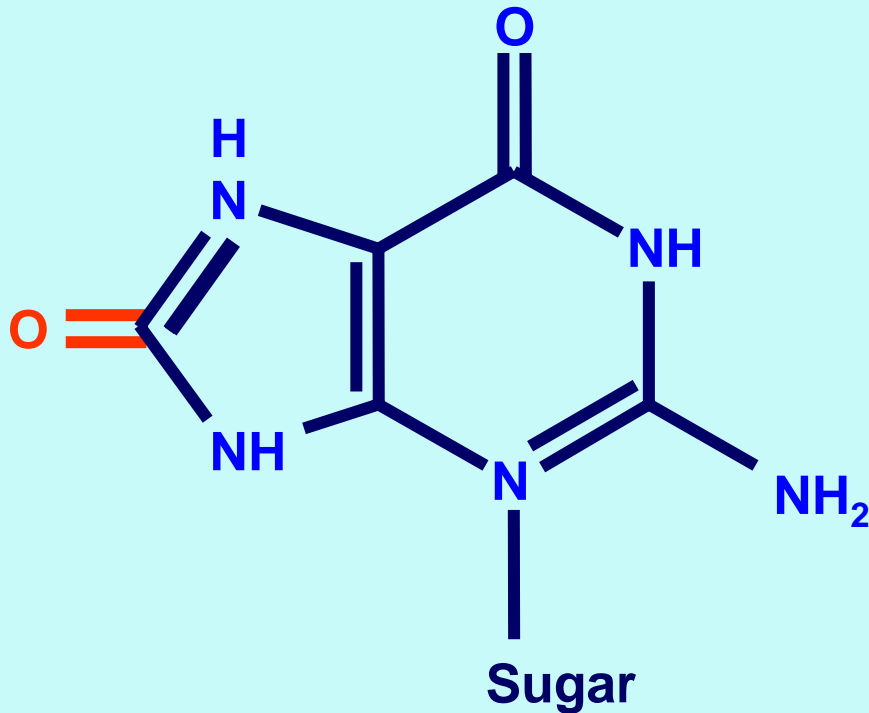
ROS Damage to Lipids



Lipid peroxidation - radicals react with lipids to yield modified lipids that can react with other membrane components

Adverse effects on membranes - reduced fluidity, decreased activity of membrane proteins, altered membrane permeability

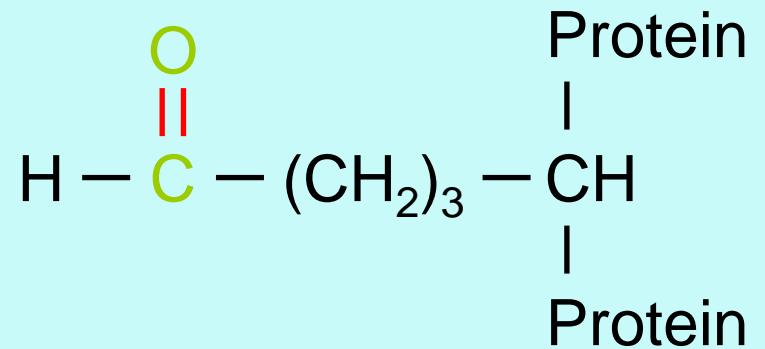
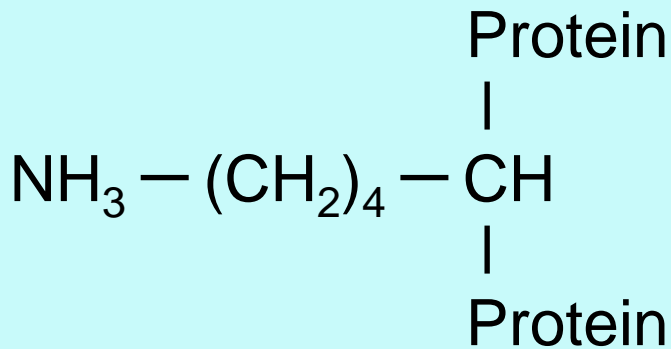
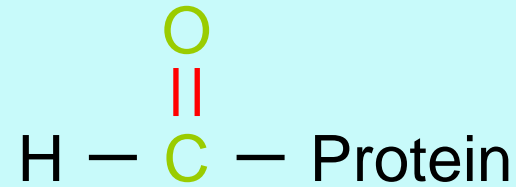
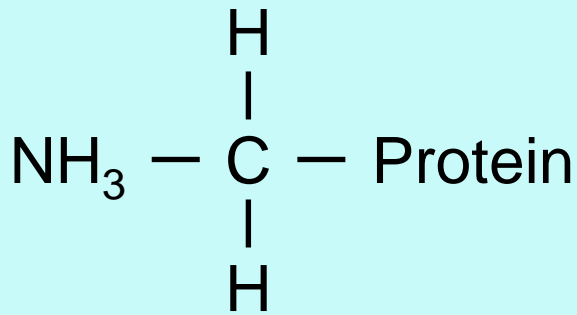
ROS Damage to DNA



8-Oxo-guanosine

DNA oxidation - chemical modification of bases in DNA
Alters coding potential for replication and transcription

ROS Damage to Proteins



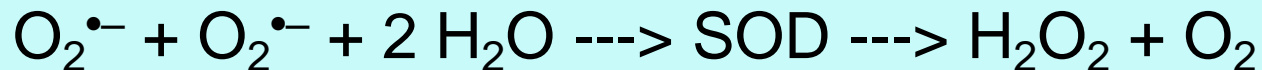
Protein oxidation - chemical modification, carbonylation
Modifications alter function and trigger degradation

Oxidative Stress Response

Cells constitutively express proteins that inactivate ROS

Cells induce expression of proteins that inactivate ROS

Superoxide dismutase (SOD)



Catalase



Glutathione (GSH) and glutathione peroxidase (GPX)



Antioxidants (vitamins C and E) can neutralize radicals

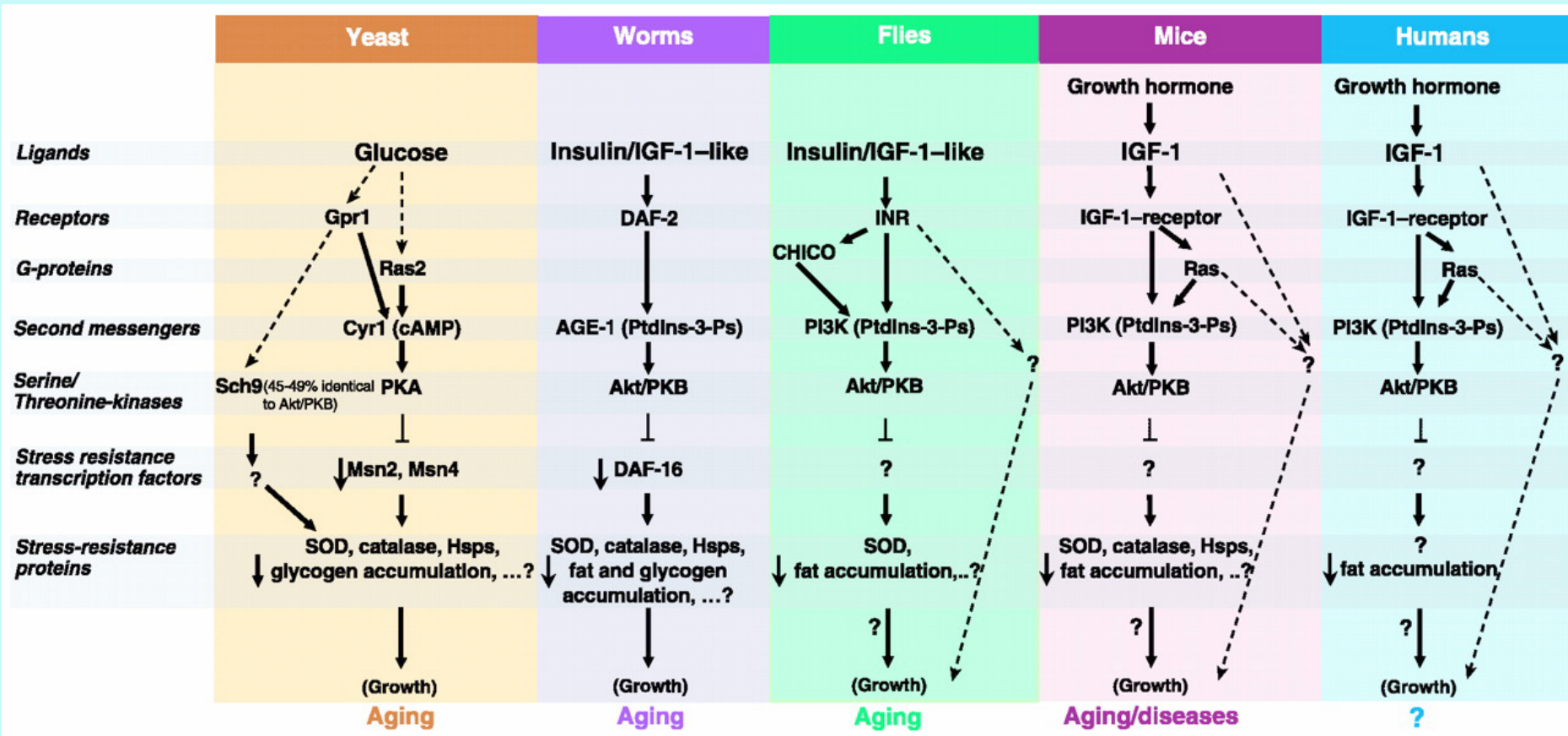
Oxidative Stress and Life Span

Drosophila that overexpress SOD2 (Cu, Zn SOD) and catalase show less oxidative damage to proteins and DNA.

Drosophila that overexpress SOD2 and catalase have a life span extended by approximately 1/3 compared to control flies (Orr and Sohal, 1994, *Science*, **263**:1128).

Chemicals that mimic the activity of catalase can extend the life span of *C. elegans*. Other mutations in *C. elegans* that extend life span require catalase activity.

Stress Response Signaling Pathways



Longo & Finch, 2003, *Science* **299**:1342

Non-Enzymatic Glucosylation

Glucose added non-enzymatically to proteins, nucleic acids

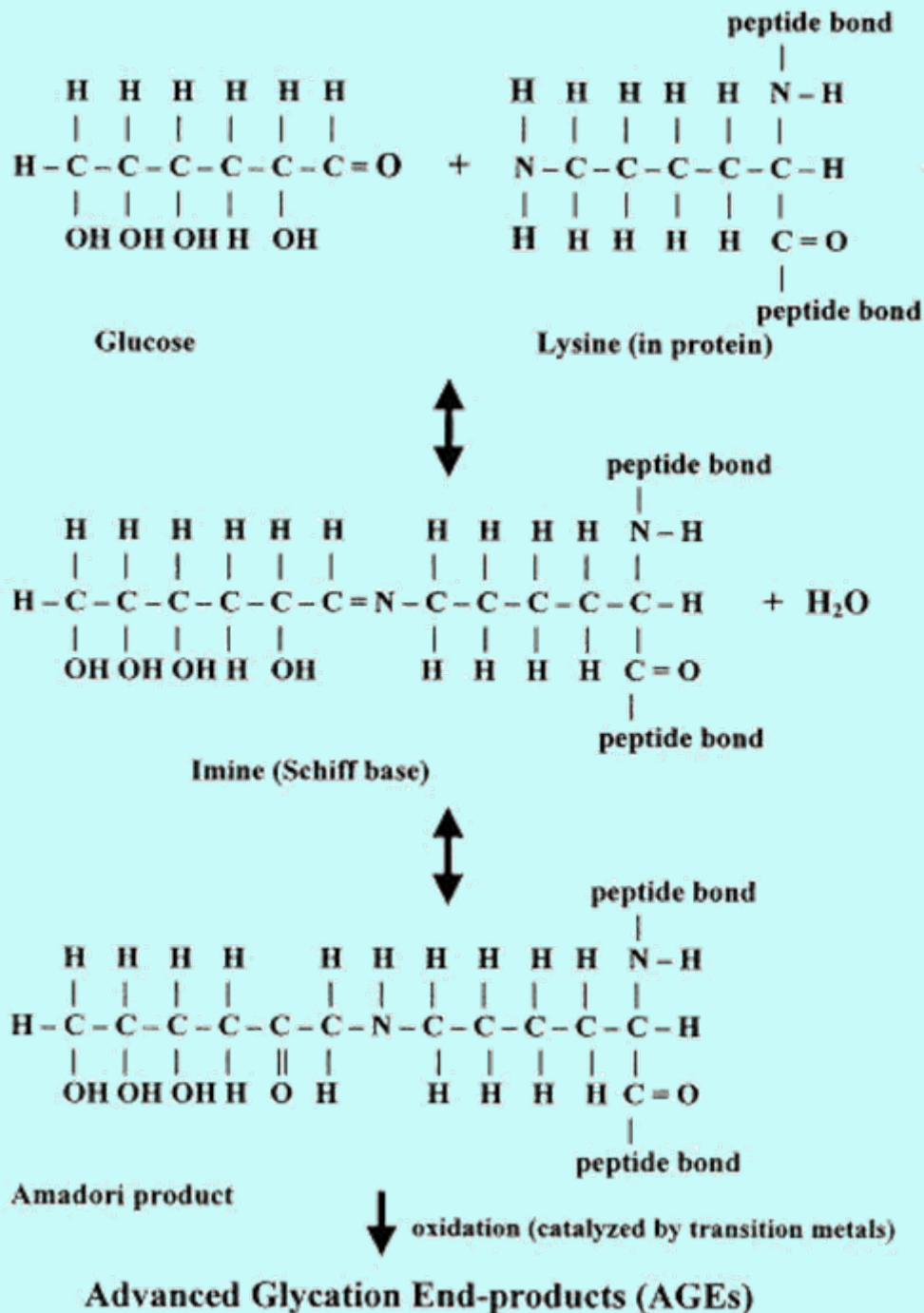
Generates Advanced Glycation End products (AGEs)

Also known as free radical addition of glucose

Modification of collagen and elastin can lead to reduced function and covalent crosslinking of these proteins, which can lead to changes in properties of connective tissue

Intracellular protein glucosylation forms modified proteins resistant to protein degradation (i.e., form residual bodies)

AGE Formation



Reducing sugars such as glucose react with protein amino groups to yield Schiff bases

Schiff bases undergo rearrangement to yield Amadori products such as ketoamine

Amadori products introduce carbonyl groups into proteins, which disrupt both structure and function.

Somatic Mutation Mechanism

Mutations in somatic cell DNA accumulate over the life span

Mutations result from exogenous or endogenous mechanisms

Mutations are passed on to progeny in mitotic cell types
(promotes “accumulation” of mutations in mitotic cell types)

Mutations may lead to alterations in:

- Chromatin structure (e.g., silencing)

- Promoter function (either increase or decrease)

- RNA processing, stability, or export

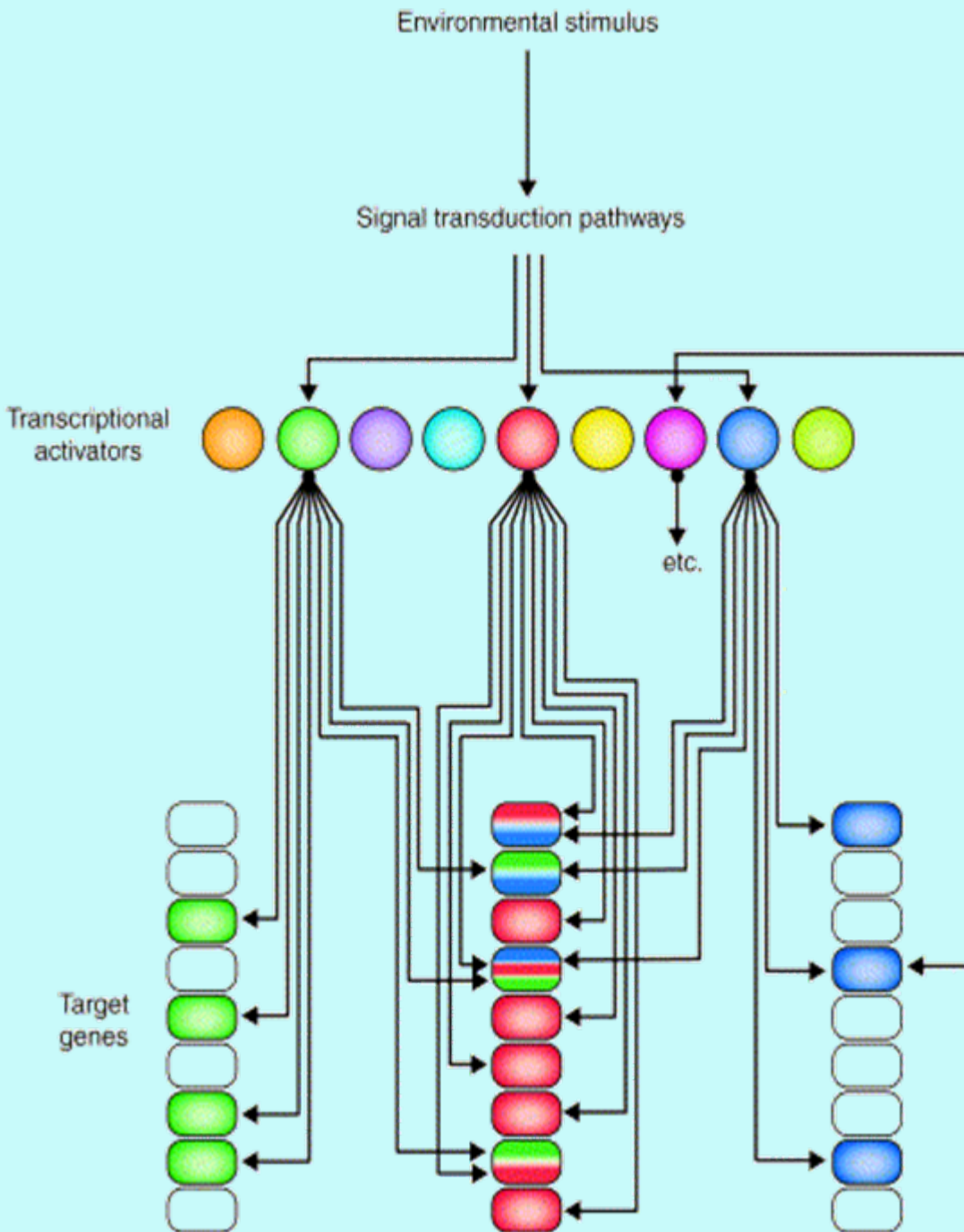
- Translation (e.g., rate and fidelity)

- Protein function, targeting, or stability

Gene Dysregulation Theory

Complex networks of regulatory pathways are sensitive to mutations and metabolic errors that accumulate over the life span and have a disproportionate effect on these pathways

Wyrick and Young, 2002, *Curr. Opin. Genet. Dev.* **12**:130-136



Genome Integrity Mechanism

Telomere - DNA/protein complex at chromosome end

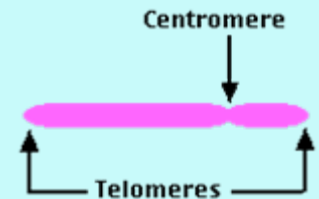
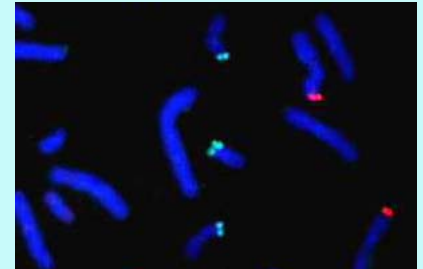
Sequence - repeated short oligonucleotide (hexameric TTAGGG repeat in human)

Length - 10^3 - 10^5 bp, depending on species

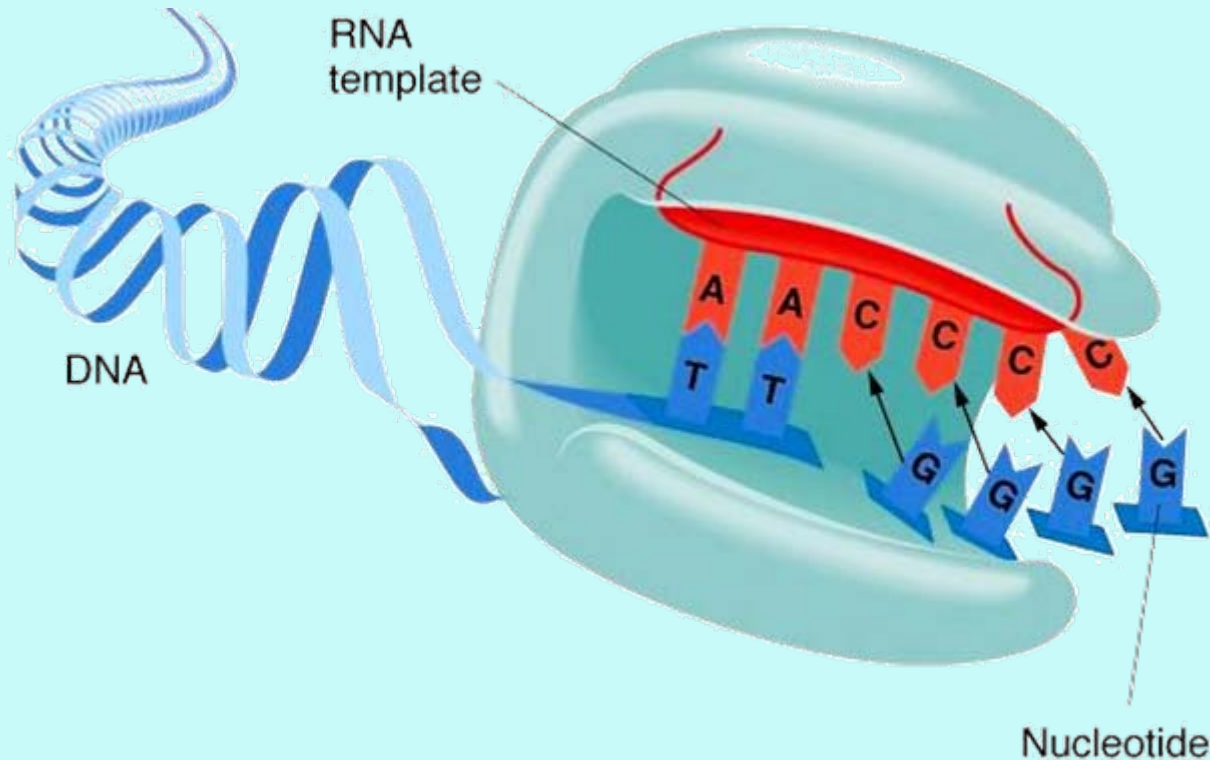
T-loop structure - free single-strand 3'-end forms triple helix loop stabilized by telomere associated proteins

Protects chromosome ends from nucleolytic attack, non-homologous end joining (NHEJ), etc.

Attrition - telomeres shorten with each cell cycle because the lagging strand can't be replicated by DNA polymerase



Telomerase

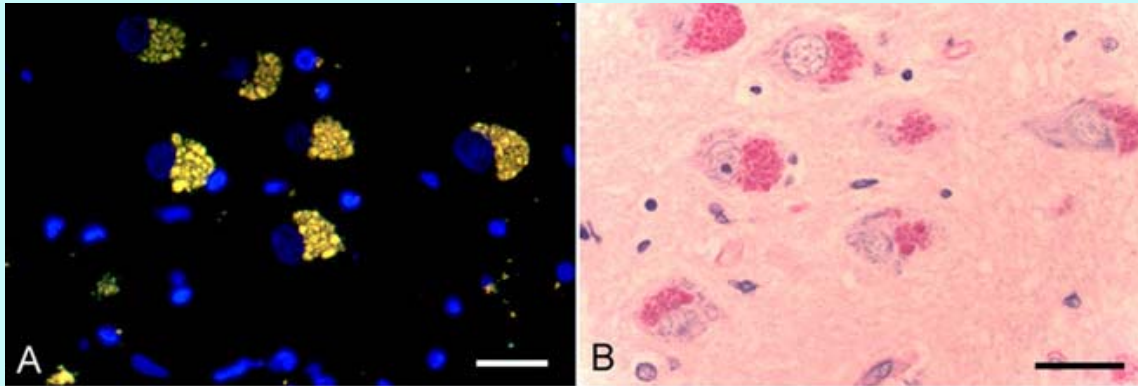


Telomerase - ribonucleoprotein complex

Catalyzes lagging strand end-replication by reverse transcription (RNA used as template for synthesis)

Telomerase - functional in germ and certain stem cells

Defect Accumulation Theory



Lipofuscin in neurons
in human brain

Gray & Woulfe, 2 Feb
2005, SAKE

Aberrant molecules fail to be degraded and accumulate

Lipofuscin - classic "age pigment" observed histologically

Lipofuscin - complex mixture of modified proteins, lipids, metals, small molecules that is resistant to degradation

Residual bodies - store lipofuscin and other substances

Incomplete degradation of mitochondria may form lipofuscin

Failed degradation mechanisms may lead to cell dysfunction

Apoptosis

Apoptosis - programmed cell death

Apoptosis is regulated by endogenous & exogenous pathways

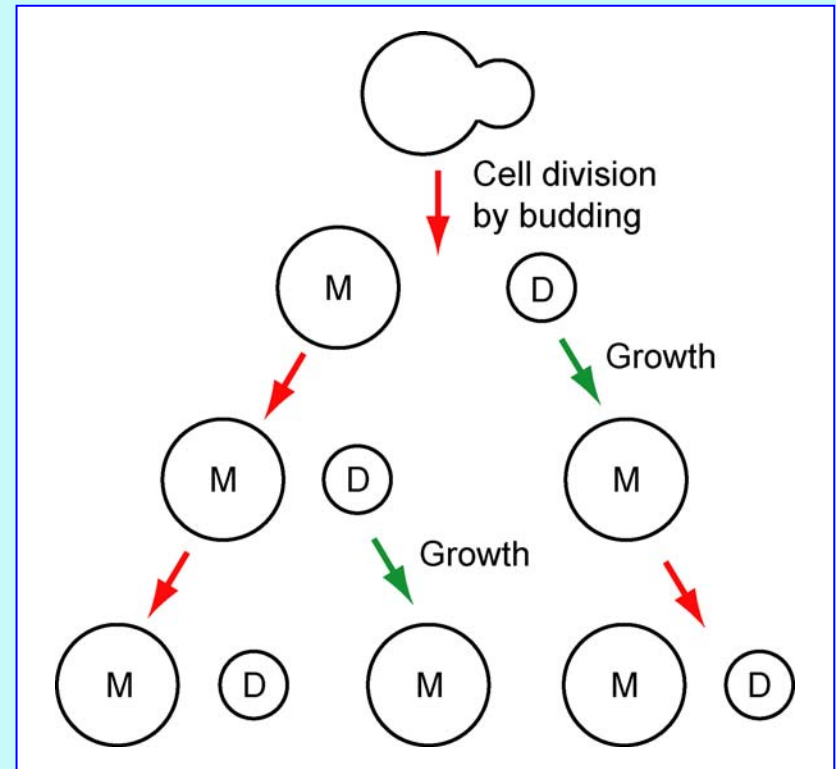
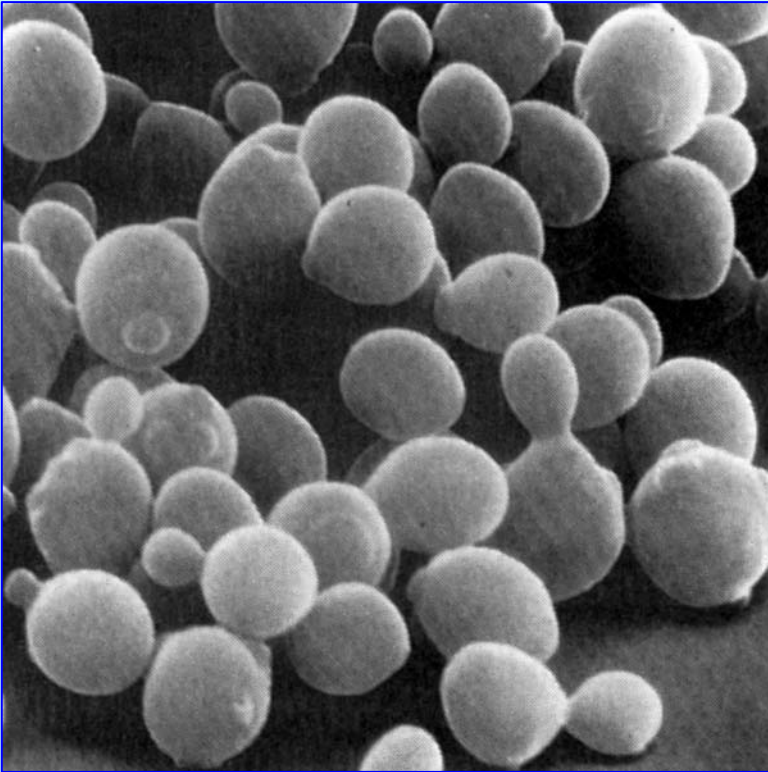
Endogenous pathway - cytochrome c release by mitochondria

Mitochondria damaged by oxidative stress have been implicated in the initiation of apoptosis

Apoptosis is also mediated by a p53 dependent pathway that senses damage to DNA (e.g., double strand breaks)

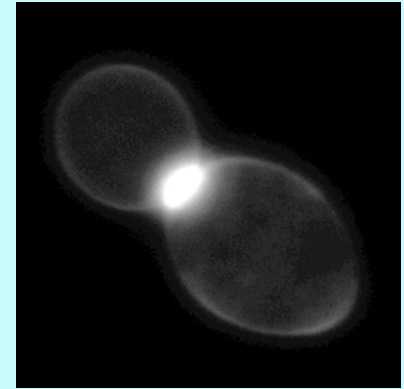
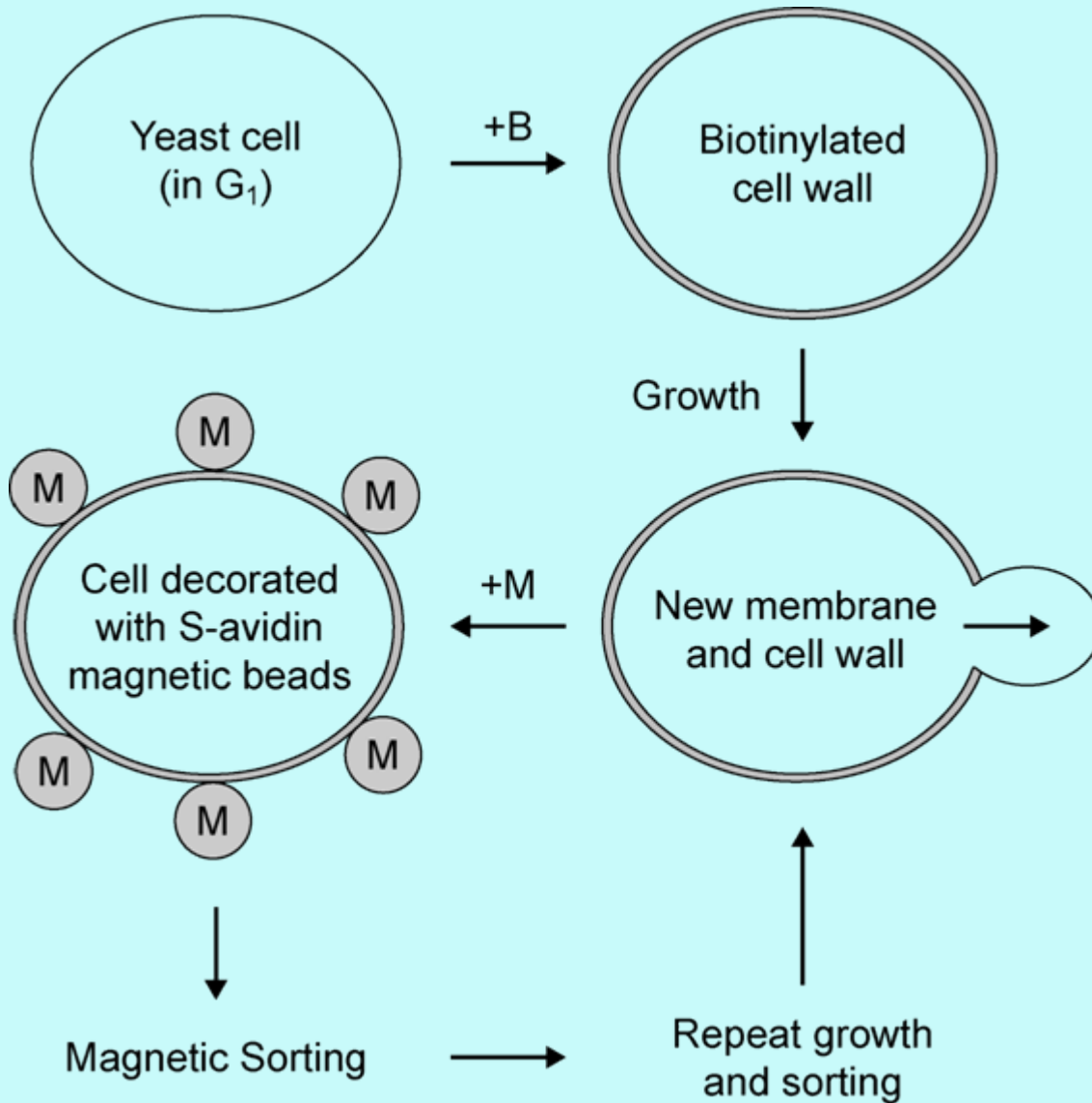
Inappropriate apoptosis may be the result of impaired regulation of pro- and anti-apoptotic pathways in old cells

Cell Division in Budding Yeast

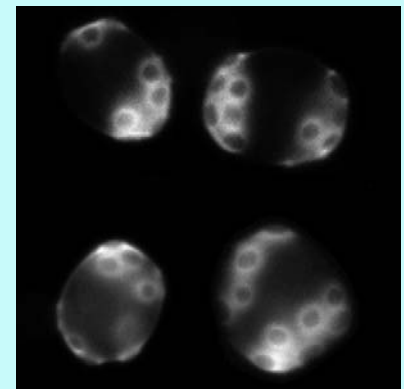


Budding is an asymmetric cell division process
Mother (M) cells give rise to daughter (D) cells

Isolation of Old Yeast Cells

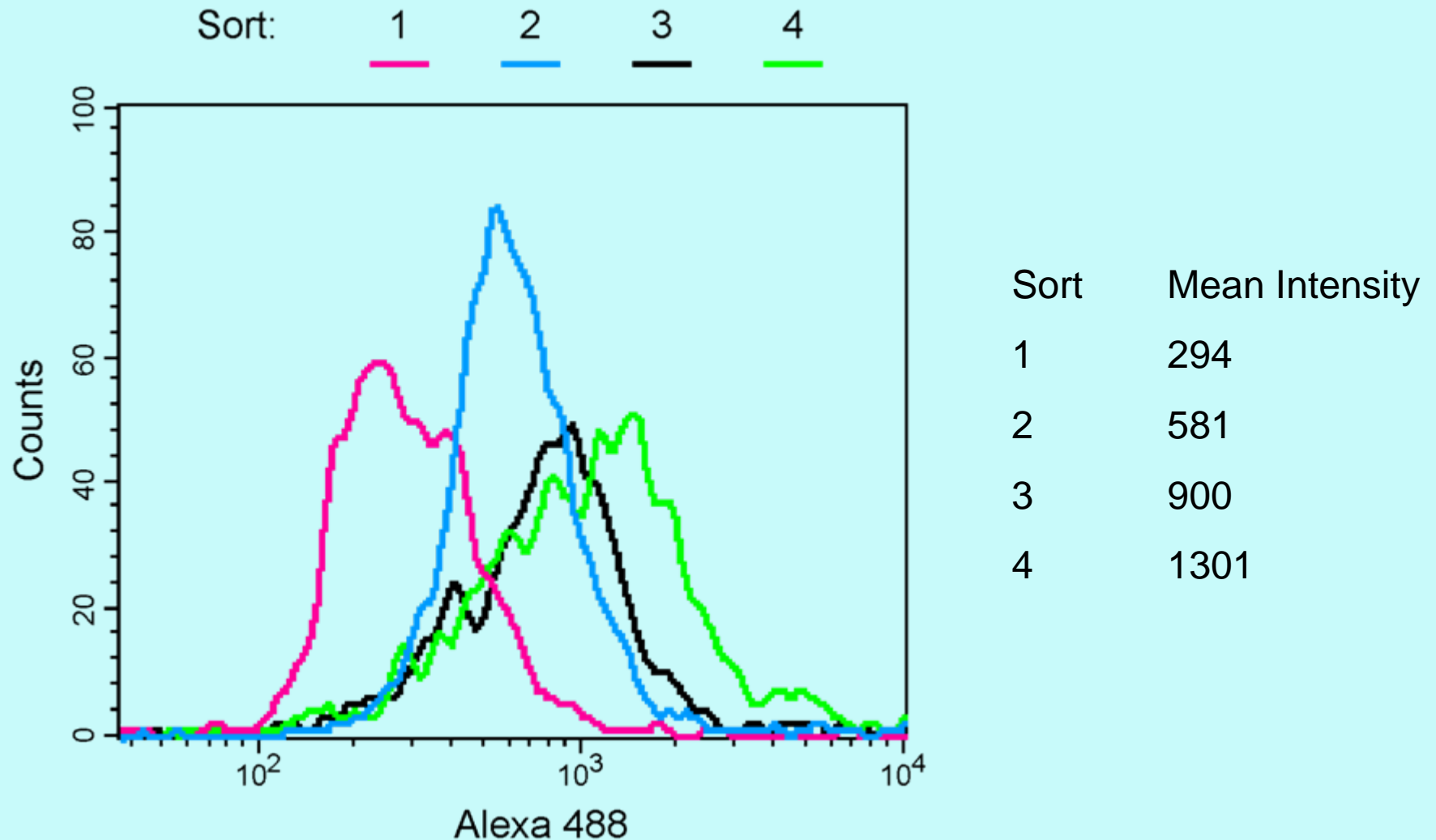


Bud ring in mitotic cell



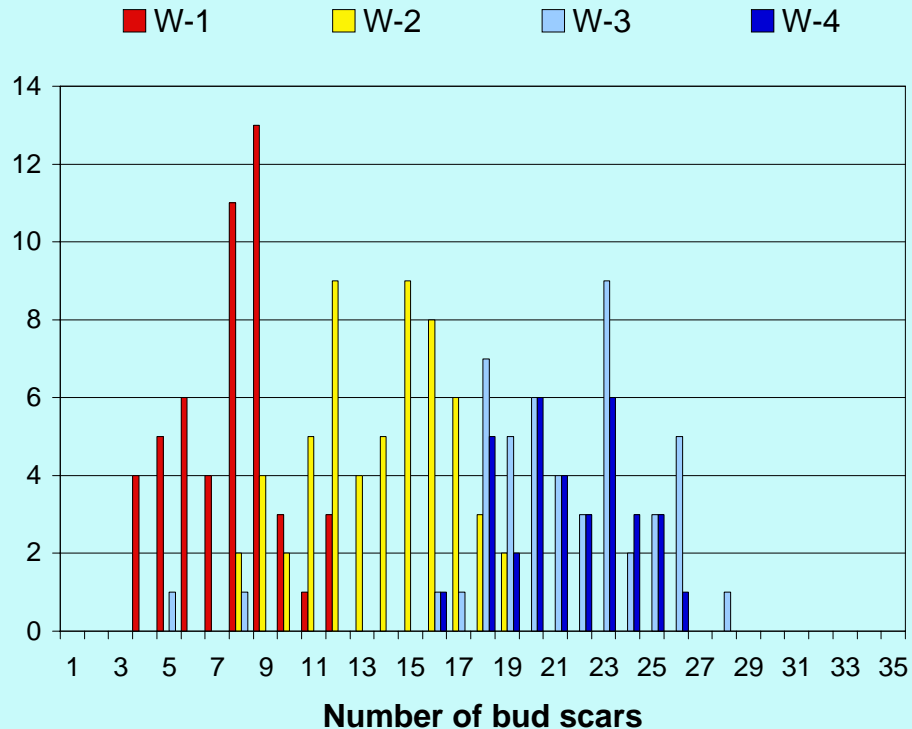
Bud scars in old cells

Serial Sorting - Flow Cytometry

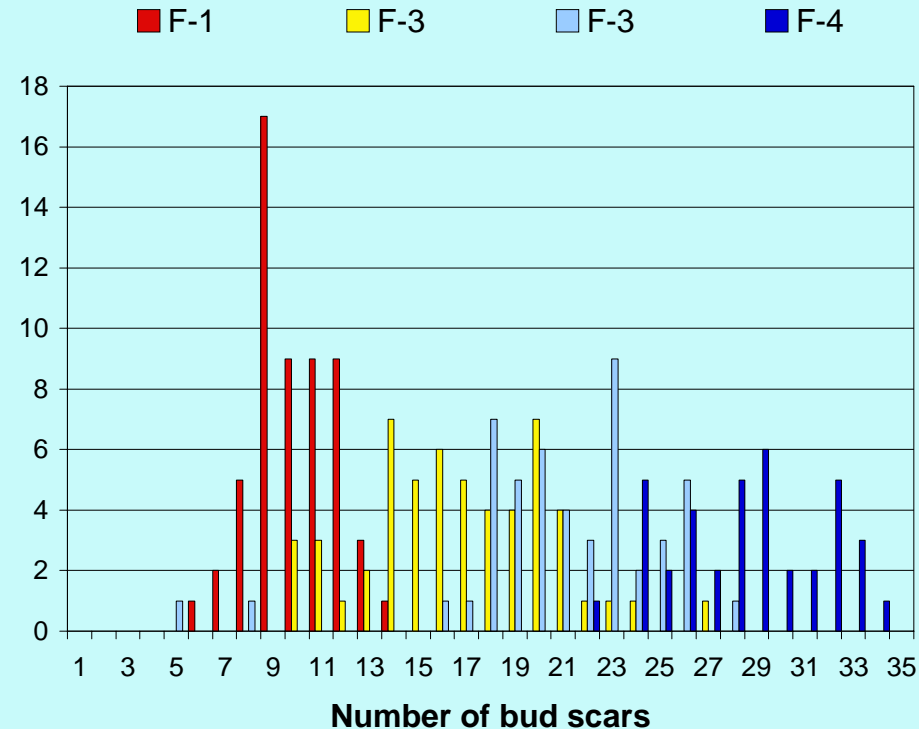


Serial Sorting - Bud Scar Counts

W303 (WT)



fob1 Δ (long-lived)



Old Yeast Cell

QuickTime™ and a
Sorenson Video 3 decompressor
are needed to see this picture.

Bud scars stained with WGA-Alexafluor488

Acknowledgements

Alaric Falcon

Natalie Rios

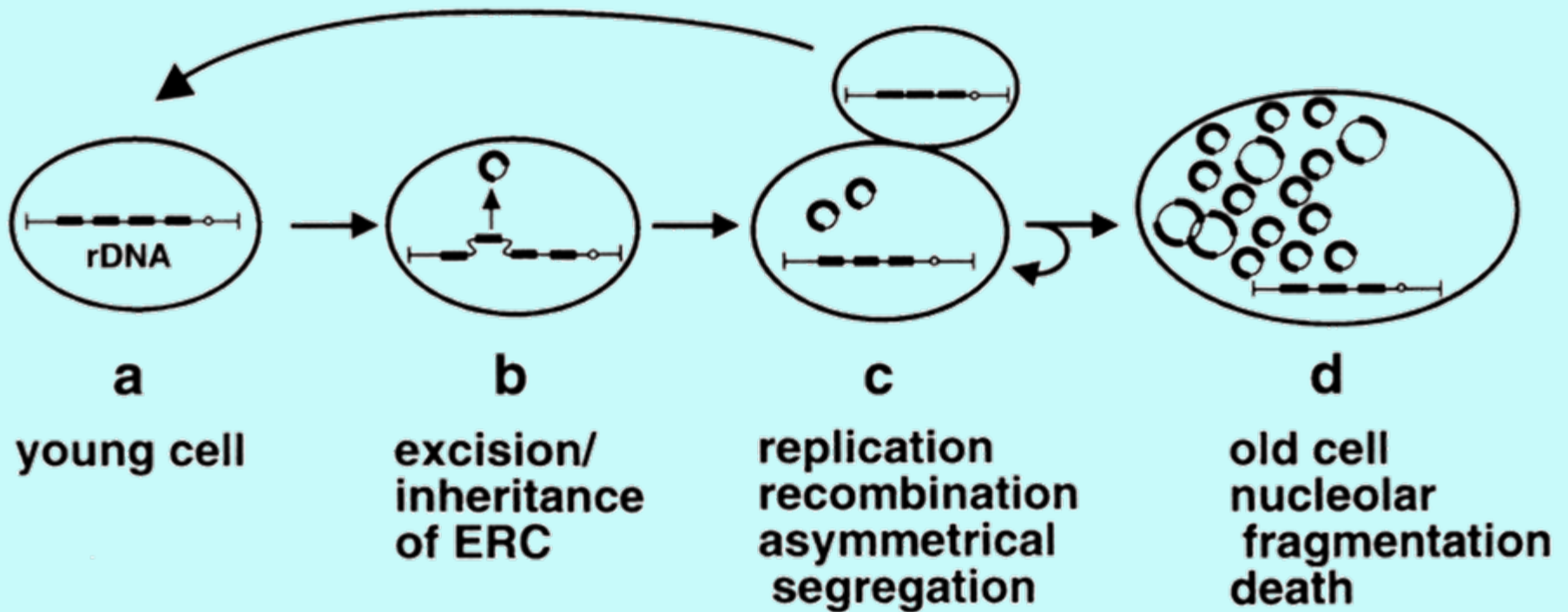
Ellison Medical Foundation

NIH NIA R21

Yeast - Good Model for Aging in Cells (as well as aging in cellars)



ERC Model



Sinclair & Guarente, 1997, *Cell* **91**:1033

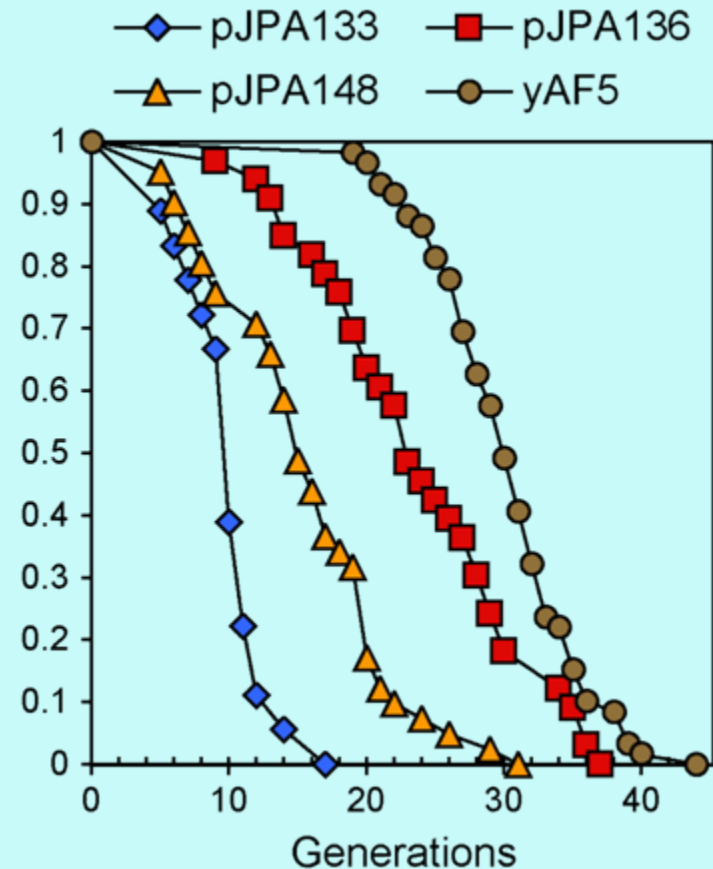
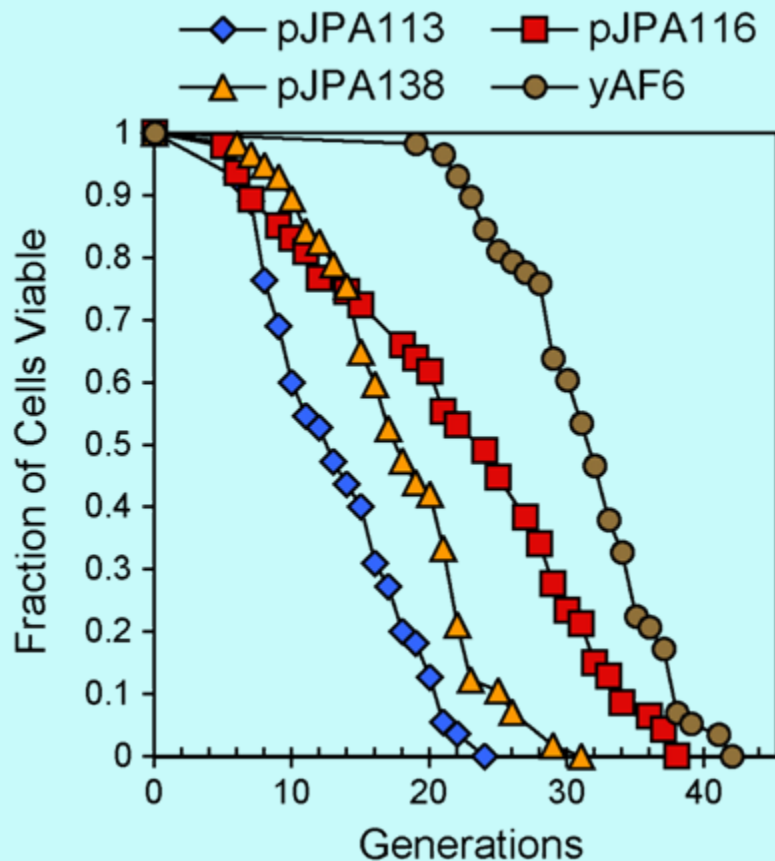
Recombinant Plasmids

Diamonds - *ARS1*

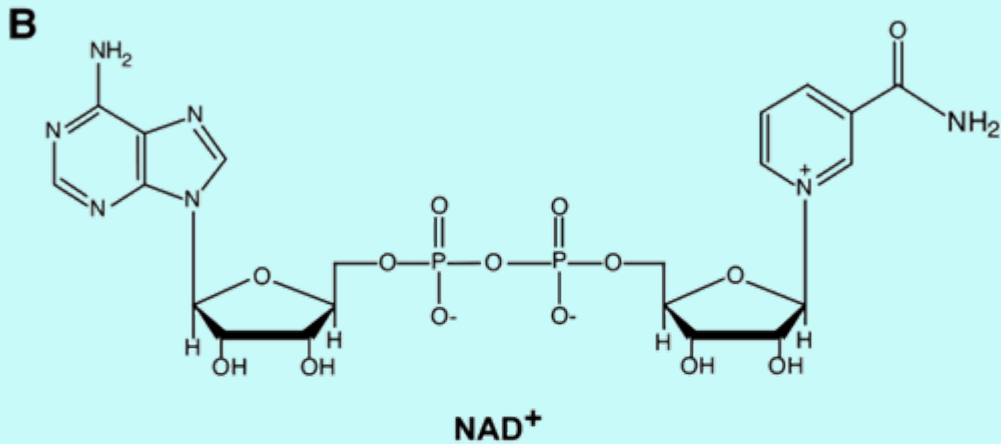
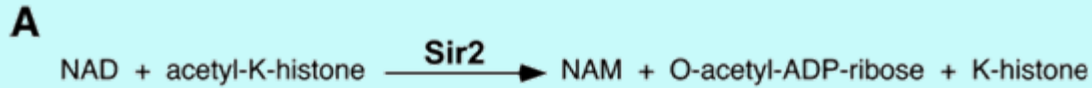
Triangles - 2 μ ori

Squares - *ARS1*, *CEN4*

Circles - no plasmid



Acetylation/Deacetylation Circuit



Acetate + CoA + ATP

Acetyl-CoA
Synthetase

K-histone + Acetyl-CoA

Histone
Acetyl
Transferase
(HAT)

Acetyl-K-histone + CoA

